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The use of DNA markers in the pre-clinical diagnosis of familial adenomatous polyposis in families in South Africa

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Haplotype association studies were performed in 10 unrelated South African families and 1 German immigrant family with familial adenomatous polyposis (FAP). Three DNA probes, recognising five restriction fragment length polymorphisms (RFLPs) around the gene locus for FAP on chromosome 5q, were used. The RFLP analysis was informative or partially informative in all the families studied. Five haplotypes were found to segregate with the disease locus. The predominant association of two of these haplotypes with FAP in the South African families suggests that two mutations may cause the disease in about 70% of families in this population. Meiotic recombination events were detected between the FAP gene and probe M4 (D5S6), but not probes Pi227 (D5S37) and C11p11 (D5S71). Haplotype analysis allowed the pre-clinical diagnosis of FAP in 5 subjects.

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Familial adenomatous polyposis (FAP) is an autosomal dominant genetic disease. In their teens patients characteristically develop many adenomatous polyps in the large bowel. Symptoms of bleeding and diarrhoea usually appear about 10 years later when patients are in their 30s. Virtually 100% of untreated sufferers develop carcinoma of the large bowel; this can be prevented in almost all cases by removal of the colon before cancer develops. The absence of rectal polyps at middle age does not mean that the disease is absent, as occasionally polyps only appear later. Potentially affected relatives of patients require regular endoscopic examination of the rectum in order to screen for the disease. As these examinations are unpleasant, patients are often understandably reluctant to undergo regular examinations that must be continued for several decades. With the recent identification[1,2] and characterisation[3,4] of the gene which causes FAP, it was hoped that it would be possible to screen potentially affected individuals for a nearby informative marker sequence and, eventually, the responsible mutation. This would allow a pre-clinical diagnosis to be made early with a single blood test, and obviate the need for repeated endoscopic examinations.

Polymorphic DNA probes which are closely linked to the FAP gene have been used to trace the defective gene in affected families.[5,6] Lod scores of 7,85 and 3,26 at recombination fractions of 0,05 and 0,0 were reported in linkage studies with FAP for probes Pi227 and C11p11, respectively.[1,5] Both these probes have been successfully used in pre-clinical diagnosis of FAP.[7-9] In this study we assessed the value of these probes, as well as probe M4 (D5S6) originally developed by us,[10] in the pre-clinical diagnosis of FAP in 11 affected families. A study of haplotype associations with FAP in the families has shown a predominant association of two haplotypes with the disease.

Material and methods

Patients and families

Blood samples were collected from 123 members of 10 South African families and one white German immigrant family. Of the South African families, 9 were white and 1 was of mixed ancestry. Histopathological proof of FAP was present in several members of each family. None of the family units studied was known to be related at the level of first or second cousin. The subjects were designated as affected or unaffected according to the findings of rigid or flexible sigmoidoscopy, colonoscopy or colectomy, supported by histopathological examination of mucosal specimens where necessary. Subjects with an endoscopically normal rectum after the age of 45 were empirically regarded as unaffected. This was thought reasonable in view of the findings of Murday and Slack.[11] Blood was taken with the informed consent of the patient and ethical approval of the appropriate institutions.

DNA preparation and haplotype analysis

DNA was prepared from blood samples by a standard method.[12] Aliquots of DNA (5 µg) were digested with the appropriate restriction enzymes, size-separated on agarose gels, transferred to nitrocellulose or nylon membranes and hybridised with radio-labelled probes according to the methods of Southern.[13] The probes were labelled *in vitro* by oligonucleotide radiolabelling to a specific activity of 10^9 cpm/µg DNA. The three polymorphic DNA probes used were the following: (*i*) M4 (D5S6), a 7,6 kb BamHI fragment in L47.1;[10] (*ii*) Pi227 (D5S37), a 0,9 kb HindIII/EcoRI fragment in PiAN7;[14] and (*iii*) C11p11 (D5S71), a 3,6 kb EcoRI fragment in pUC8.[1] The restriction fragment length polymorphisms (RFLPs) that these probes recognise and the restriction enzymes used are reported in Table I. FAP-associated haplotypes for all subjects were deduced by segregation analysis or by homozygosity for all the RFLPs.

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Table I. Allele sizes recognised on Southern blot analysis at five polymorphic loci near the FAP gene locus

Polymorphism			
Probe	Enzyme	Allele	Size (kb)
M4	BamHI	1	11,0
		2	9,6
		3	7,6
C11p11	TaqI	1	4,4
		2	3,9
Pi227	PstI	1	4,3
		2	3,0
	BclI	1	8,6 + 1,2
		2	3,0
		3	1,8 + 1,2
	MboI	1	0,55
		2	0,45

Results

We used five RFLPs to trace the inheritance of the FAP gene in the 11 unrelated families. The three probes and restriction enzymes used to recognise the RFLPs on chromosome 5q are summarised in Table I. All variants showed autosomal codominant inheritance in the families and five haplotypes associated with FAP could be identified. Two of these haplotypes were predominantly associated with the disease, while three were identified in single families. The different haplotypes identified in the families studied are shown in Table II. The haplotypic arrangement 2212 was associated with the FAP mutation in the German immigrant family, while haplotype 2232 showed disease association in 5 South African families, including the one of mixed ancestry. The frequencies of haplotypes for 49 normal chromosomes were deduced from the patients and their unrelated spouses. In addition to the five FAP-associated haplotypes another seven were found in the normal population. Although a small number of unrelated FAP cases were haplotyped, it is clear that both the frequency and distribution of haplotypes differ significantly between the normal and FAP samples. Analyses of 77 phase-known meioses have shown that probe M4 was involved in 5 recombination events, while no crossovers were identified between FAP and Pi227 or between FAP and C11p11. For this reason, and since M4 did not distinguish a specific FAP-associated haplotype, the additional haplotypes identified in normal chromosomes by using this marker are not given in Table II.

Table II. Haplotypes of the FAP gene observed with probes C11p11 and Pi227 in the study population

Probe/restriction enzyme				No. of haplotypes	
C11p11	Pi227			FAP-associated	Non-FAP associated*
TaqI	PstI	BclI	MboI	(*N* = 11)	(*N* = 49)
2	2#	2	1	1	19 (38%)
2	2	3	2	5	14 (29%)
2	2#	1	2	1	5 (10%)
2	1	3	2	3	3 (6%)
1	2	1	2	0	2 (4%)
1	2	3	2	0	1 (2%)
1	2	3	1	0	1 (2%)
2	2	3	1	0	1 (2%)
2	2	2	2	0	1 (2%)
2	1	2	1	0	1 (2%)
1	2	2	1	0	1 (2%)
1	1	3	2	1	0

* Haplotype frequencies in normal chromosomes are given in brackets.
# Unambiguous assignment of PstI RFLP alleles to specific haplotypes was not possible in some of the individuals tested.

The DNA analysis was informative in all the families studied, although only partly informative in 1. Of the individuals studied, 27 were normal spouses, 52 were affected and 44 were at risk for FAP. The DNA diagnosis was uninformative in 4 of the 44 individuals at risk, while in the remainder, affected haplotypes were assigned to 13 and normal haplotypes to 27. To establish how accurately identification of the disease-associated haplotype predicted the inheritance of FAP, 7 individuals with this haplotype were examined clinically and in 5 FAP was proven histopathologically. Two subjects, aged 73 and 34 years respectively, were also shown to carry the haplotype, although no signs of FAP could be detected. An example of how DNA analysis was used to predict FAP in an affected family is shown in Fig. 1.

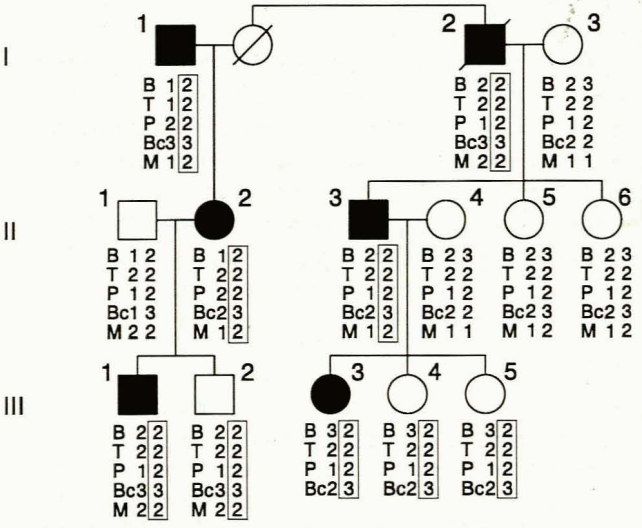

Fig. 1. The segregation of proven FAP (shaded squares and circles) and RFLPs of the disease gene in a family. The affected haplotype is boxed. In individuals III-2, III-4 and III-5 the finding of the FAP haplotype suggested the presence of FAP before clinical examination. In individual III-2 FAP was subsequently diagnosed by means of a colonoscopy. Individuals III-4 and III-5 have not yet been examined clinically.

Discussion

The aim of this study was to assess how accurately one was able to diagnose FAP in members of affected South African families, using DNA probes that identify RFLPs close to the FAP gene. The potential clinical value of using linked DNA probes to analyse an individual's risk of having FAP has been examined by several groups.[7-9] Probes Pi227 and C11p11 are closely linked to the FAP gene and have been successfully used in pre-clinical diagnosis.[1,5] Our data, obtained from 123 individuals in 11 families unrelated to the second degree, provided further evidence that probes Pi227 and C11p11 are potentially useful tools for the identification of affected family members without clinical signs of FAP. The haplotype analysis assigned high risk to 13 subjects. Of the

7 subjects who were subsequently examined clinically, 5 were shown to be affected. Endoscopic examination of the two remaining individuals showed them to be unaffected. The 'affected' haplotypes might be present in them because of recombination events, incomplete penetrance or because the subjects have yet to develop polyps. These different possibilities are discussed by Mareni and coworkers,[9] who describe a similar discrepancy between clinical phenotype and DNA characteristics. The degree of usefulness of different probes used in pre-clinical diagnosis, as well as the disease-associated chromosomal arrangements identified, differ between populations.[1,15] In the Dutch population, less heterozygosity was detected by C11p11 than in the English population.[5] As would be expected, the degree of heterozygosity found in our population is closer to that of the Dutch population.

We did not identify any crossovers between the FAP gene and either probes Pi227 or C11p11, but our probe M4 was involved in 5 recombination events (77 meioses analysed). In a study by Meera Khan and collaborators[15] in 1987, the genetic distance between the FAP locus and M4 was estimated to be about 25 cM. Because of low informativeness, the data obtained from two additional 5q probes developed during this study are not reported here.[16]

Using four RFLPs identified by Pi227 and C11p11, we have determined the haplotypic arrangements of both normal and FAP associated alleles in 119 of the 123 family members. A total of eleven different haplotypes were identified in 49 normal chromosomes. Four of these, as well as one additional haplotype, were found to segregate consistently with FAP in the 11 families studied. The association of predominantly two haplotypes with FAP in the South African families further indicated that two different gene mutations may cause the disease in about 70% of families in this population. This suggests the presence of specific founder genes for FAP in some of the affected families studied. This phenomenon has also been shown for other genetic diseases in isolated populations.[17,18] We have previously confirmed the proposed 'founder effect' for familial hypercholesterolaemia in South African Afrikaners[19] by haplotype studies[20] and subsequent mutation detection.[21]

The recent identification of the FAP gene enables the detection of disease-related mutations and should eventually enable the development of rapid DNA-based methods for diagnosis. The specific FAP-associated haplotypes found to segregate in the families analysed by us will facilitate the detection of mutations. This analysis provided an indication of the number of different mutations present in South African patients and confirmed that the FAP phenotype segregating in the families is caused by defects in the gene analysed. Although our results suggest that four different mutations may cause FAP in the South African families studied, there is the possibility that only one mutation exists and has become associated with different RFLP haplotypes by recombination events. It is, however, more likely that the prediction of five mutations (including one in the German immigrant family) is a minimum estimate, since the haplotypes may be genetically heterogeneous, with several mutations causing FAP present on the same chromosomal background. Studies are currently in progress to identify the gene mutations underlying FAP in the South African population, which may confirm or refute the founder hypothesis.

Our results have shown that closely-linked polymorphic DNA markers can be used to diagnose FAP in local families before clinical proof is available. The problem of crossing over between markers and the FAP gene can in future be circumvented by the use of additional markers on either side of the gene, thereby making the predictions totally reliable.[7] Recently identified polymorphisms closer to and within the FAP gene[22,23] are currently being investigated for this purpose. Ultimately, the identification of the specific mutations that cause the disease may enable us to predict or exclude FAP reliably with a single test.

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REFERENCES

1. Bodmer WF, Bailey CJ, Bodmer J, Bussey HJR, Ellis A, Gorman P, *et al.* Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987; **328:** 614-616.
2. Leppert M, Dobbs M, Scambler P, O'Connell P, Nakamura Y, Stauffer D, *et al.* The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 1987; **238:** 1411-1413.
3. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991; **66:** 589-600.
4. Kinzler KW, Nilbert MC, Vogelstein B, Bryan TM, Levy DB, Smith KJ, *et al.* Identification of a gene located at chromosome 5q21 that is mutated in colorectal cancers. *Science* 1991; **251:** 1366-1370.
5. Meera Khan P, Tops CMJ, Van den Broek M, Breukel C, Wijnen JTh, Oldenburg M, *et al.* Close linkage of a highly polymorphic marker (D5S37) to familial adenomatous polyposis (FAP) and confirmation of FAP localization on chromosome 5q21-22. *Hum Genet* 1988; **79:** 183-185.
6. Varesco L, Thomas HJW, Cottrell S, Murday V, Fennell SJ, Williams S, *et al.* CpG island clones from a deletion encompassing the gene for adenomatous polyposis coli. *Proc Natl Acad Sci USA* 1989; **86:** 10118-10122.
7. Dunlop MG, Wyllie AH, Steel CM, Piris J, Evans HJ. Linked DNA markers for presymptomatic diagnosis of familial adenomatous polyposis. *Lancet* 1991; **337:** 313-316.
8. Koorey DJ, McCaughan GW, Trent RJ, Gallagher ND. Risk estimation in familial adenomatous polyposis using DNA probes linked to the familial adenomatous polyposis gene. *Gut* 1992; **33:** 530-534.
9. Mareni C, Stella A, Origone P, Susca F, Montera MP, Lonoce A, *et al.* Linkage studies in Italian families with familial adenomatous polyposis. *Hum Genet* 1993; **90:** 545-550.
10. Dietzsch E, Retief AE, Kotze MJ, Warnich L, Nicholson DL, Fox MF, *et al.* An anonymous human single copy genomic clone, D5S6 (M4) on chromosome 5 identifies a three allele RFLP. *Nucleic Acids Res* 1986; **14:** 1923.
11. Murday V, Slack J. Inherited disorders associated with colorectal cancer. *Cancer Surv* 1989; **8:** 139-157.
12. Kunkel LM, Smith KD, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, *et al.* Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 1977; **74:** 1245-1249.
13. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975; **98:** 503-517.
14. Stewart GD, Bruns GAP, Wasmuth JJ, Kurnitt DM. An anonymous DNA segment (π227) maps to the long arm of human chromosome 5 and identifies a BstXI polymorphism (D5S26). *Nucleic Acids Res* 1987; **15:** 3939.
15. Meera Khan PM, Tops C, Van den Broek MH, Breukel C, Oldenburg M, Wijnen JTh, *et al.* Linkage between adenomatous polyposis coli (APC), D5S6 and FMS on chromosome 5. *Cytogenet Cell Genet* 1987; **46:** 661.
16. Grobbelaar JJ. Die gebruik van restriksie fragment lengte polimorfismes as genetiese merkers vir die vroeë diagnose van familiële kanker van die kolon. M.Sc. Thesis, University of Stellenbosch, 1992.
17. Navon R, Kolodny EH, Mitsumoto H, Thomas GH, Proia RL. Ashkenazi-Jewish and non-Jewish adult G_{M2} gangliosidosis patients share a common genetic defect. *Am J Hum Genet* 1990; **46:** 817-821.
18. Hobbs HH, Russell DW, Brown MS, Goldstein JL. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet* 1990; **24:** 133-170.
19. Seftel HC, Baker SG, Sandler MP, Forman MB, Joffe BI, Mendelsohn D, *et al.* A host of hypercholesterolaemic homozygotes in South Africa. *BMJ* 1980; **281:** 633-636.
20. Kotze MJ, Langenhoven E, Retief AE, Seftel HC, Henderson HE, Weich HFH. Haplotypes identified by 10 DNA restriction fragment length polymorphisms at the human low density lipoprotein receptor gene locus. *J Med Genet* 1989; **26:** 255-259.
21. Kotze MJ, Langenhoven E, Warnich L, Du Plessis L, Retief AE. The molecular basis and diagnosis of familial hypercholesterolaemia in South African Afrikaners. *Ann Hum Genet* 1991; **55**: 115-121.
22. Spirio L, Joslyn G, Nelson L, Leppert M, White R. A CA repeat 30-70 kb downstream from the adenomatous polyposis coli (APC) gene. *Nucleic Acids Res* 1992; **19:** 6348.
23. Kraus C, Ballhausen WG. Two intragenic polymorphisms of the APC-gene detected by PCR and enzymatic digestion. *Hum Genet* 1992; **88:** 705-706.

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